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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/439,534	11/12/1999	SIMON GEIR MOLLER	2312-103	1755

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EXAMINER
MEHTA, ASHWIN D

ART UNIT	PAPER NUMBER
1638	

DATE MAILED: 02/25/2002

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/439,534	MOLLER ET AL.	
	Examiner	Art Unit	
	Ashwin Mehta	1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 December 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *39-47,59,60 and 72* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *39-47,59,60 and 72* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *12* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

2. The objection to claim 60 is withdrawn, in light of the claim amendment.

3. The rejection to claim 46 under 35 U.S.C. 112, 2nd paragraph, is withdrawn, in light of the claim amendment.

4. The rejection to claim 42 under 35 U.S.C. 112, 1st paragraph, is withdrawn, in light of the claim amendment.

5. The rejection to claim 45 under 35 U.S.C. 112, 1st paragraph, is withdrawn, in light of Applicants' arguments.

6. The rejection to claim 46 under 35 U.S.C. 112, 1st paragraph, is withdrawn, in light of Applicants' arguments.

7. The rejection to claims 39-44, 59, 60, and 72 under 35 U.S.C. 103(a) is withdrawn and replaced with the rejection below.

Claim Rejections - 35 USC § 112

8. Claims 39-47, 59, 60, and 72 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for vectors wherein the transcription factor and promoter of the inducible gene are based on the GVG system, and wherein the vector is a plant transformation vector, does not reasonably provide enablement for vectors comprising all transcription factors and inducible promoter systems and non-plant vectors. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The claims are broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene; or wherein the transcription factor comprises a hormone binding domain of the glucocorticoid receptor; or wherein said marker gene is under the control of a high affinity promoter and the inducible gene is under the control of a low affinity promoter, both promoters being induced by the same inducer; or wherein said recombinase comprises an N-terminal transit peptide for chloroplast targeting; or a method for excising a marker gene from the genome of a transgenic plant or plant cell, comprising transfecting a plant or plant cell with said vector; or a plant or plant cell comprising said vector.

The specification teaches the use of an inducible promoter system along with a site-specific recombination system in order to excise marker genes from transgenic plants. The exemplified induction system is the GVG inducible system. The invention comprises a vector comprising a gene of interest, a marker gene, a sequence encoding the transcription factor GVG,

and a recombinase-encoding gene under the control of an inducible promoter. Recombination sites that are recognized by the recombinase flank the portion of the vector that comprises the marker gene, a gene encoding the chimeric transcription factor GVG, and the recombinase gene. The GVG transcription factor consists of the hormone-binding domain of the rat glucocorticoid receptor, the yeast Gal4 DNA-binding domain, and the transcriptional activator from the herpes viral protein, V16. The recombinase gene is operably linked to a promoter that comprises a Gal4 upstream activating sequence. The vector is introduced into plant cells and following selection of transgenic plants or plant cells expressing the marker gene, the chemical inducer dexamethasone is applied. Dexamethasone causes a conformational change to GVG, which can then recognize and induce promoters comprising the Gal4 upstream activating sequence. The expressed recombinase excises the nucleotide sequences that are flanked by the recombination sites (page 4, line 28 to page 5, line 26; page 8, line 19 to page 9, line 12). In Example 1, the specification teaches that the GVG system removed a "stuffer" fragment that had lox recombination sites on its ends, from a LUC reporter gene in transgenic Arabidopsis seedlings. Luciferase can only be expressed after the stuffer fragment is removed. Luciferase expression was only detected in transgenic leaves that were treated with dexamethasone (page 7, lines 4-24).

However, the specification does not enable use of the claimed invention with all inducible promoters, as broadly encompassed by the claims. Page 3, lines 10-20 indicates that specific activation of transgenes at specific times, and the ability to temporally and spatially induce recombinase expression, is required to practice the invention. However, not all inducible promoter systems confer tight enough control to be useful with the claimed invention. For example, although the specification at page 5, lines 14-17 teaches that the heat-inducible

expression system of Lyznik et al. (Plant J., 1995, Vol. 8, pages 177-186) can be used, Applicants, in the paper submitted 10 December 2001, admit that this system is very leaky, and suffers from deficiencies that preclude its use in the system contemplated by the invention (response, page 14, last paragraph to page 15, first full paragraph). The specification does not teach what systems provide a tight enough control for use with the claimed invention. In the absence of further guidance undue experimentation would be required by one skilled in the art to determine which inducible promoter systems, besides the GVG system, are suitable for use with the claimed invention, including those suggested in the specification. Aoyama et al. (Glucocorticoid-inducible Gene Expression in Plants, in *Inducible Gene Expression in Plants*, Ed. P. Reynolds, CAB International, Wallingford, pages 43-59, 1999) teach that induction systems based on vertebrate steroids could become powerful tools in transgenic plants, since plant do not have natural receptors for them (page 47, first full paragraph). Aoyama et al. also teach that the hormone-binding domain of the GVG protein can be replaced by that of other hormone receptors (page 54, last paragraph). It is suggested that claim 39 be amended to indicate that the transcription factor comprises the hormone binding domain of a vertebrate steroid, the Gal4 DNA-binding domain, and a transcriptional activator, and that the transcription factor, when activated by an inducer, activates expression of the inducible gene.

Further regarding claim 47: the specification indicates that the system can be used to evict marker genes following chloroplast transformation (page 10, line 16 to page 11, line 2). The specification teaches that a transgene cassette harboring a selective marker gene is flanked by directly repeated lox sites and resides in the plastid genome. Following selection, the recombinase CRE is induced, wherein the recombinase further comprises sequences required for

chloroplast targeting. When expressed, CRE translocates to the plastids and acts upon the lox sites (page 10, lines 24 to 31).

However, it is not clear how the vector in claim 47 can be used with the system taught in specification. The specification indicates that the marker gene is located in the chloroplast genome. Parent claim 39 indicates that both the marker gene and the recombinase gene are on the same vector, and flanked by the same recombination sites. If the claimed vector were introduced into the chloroplast, then the recombinase gene would be transformed into the chloroplast as well. However, the chloroplast transit peptide has no use in such an embodiment, since the recombinase would be expressed in the chloroplast. It appears then that the vector of claim 47 does not encompass chloroplast transformation vectors. If the claimed vector were not introduced into the chloroplast, then the presence of the transit peptide would direct the recombinase into a location that does not comprise its substrate recombination sites. The vector remains in the nuclear genome, whereas the expressed recombinase gets directed to the chloroplast. The vector of claim 47 then cannot be used for the purpose taught by the specification. In the absence of further guidance, it would require undue experimentation by one skilled in the art to use the claimed invention to excise DNA sequences from a transformation vector following plant transformation, when the recombinase gene resides on the vector and when the expressed recombinase is directed to a location that separates it from its substrate recombination sites on the vector.

Further, the specification indicates that the vectors of the invention are intended for use with transgenic plants and plant cells (page 3, lines 10-13; for example), the explanation of the invention throughout the specification is in terms of its use in plants. As the specification does

not teach how to use the claimed vector in other organisms, it is suggested that claim 39 be amended by inserting the recitation --plant transformation-- in line 1 before "vector". Given the breadth of the claim, unpredictability of the art and lack of guidance of the specification as discussed above, undue experimentation would be required by one skilled in the art to make and use the claimed invention.

Claim Rejections - 35 USC § 103

9. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

10. Claims 39-44, 59, 60, and 72 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sugita et al. (U.S. Patent No. 6,326,192) in view of Aoyama et al. (Plant J., 1997, Vol. 11, pages 605-612).

The claims are broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene; or a method for excising a marker gene from the genome of a

transgenic plant or plant cell, comprising transfecting a plant or plant cell with said vector; or a plant or plant cell comprising said vector.

Sugita et al. teach a method of producing a transgenic plant free of a marker gene. The method comprises introducing a vector into a plant cell, wherein said vector comprises a gene of interest, a marker gene, and a removable DNA element that can be removed by the action of a site-specific recombinase. The marker gene and a gene encoding the recombinase are located within the removable DNA element, which is flanked by recombination sites that are recognized by the recombinase, and the sites are oriented in the same direction. Examples of site-specific recombinases that can be used include FLP, CRE, and R, and recombination sites include FRT, lox, and RS. Further, the recombinase gene is placed under the control of an inducible promoter, so that the removable DNA element comprising the marker and recombinase genes would not be excised until the recombinase gene promoter is induced, after selection of transgenic plants. Sugita et al. list several inducible promoters known in the art, including the glucocorticoid system promoter (col. 2, line 59 to col. 3, line 2; col. 4, line 47, to col. 6, line 32; col. 6, lines 25-33; col. 8, line 45 to col. 16, line 35).

Sugita et al. do not teach a vector comprising a gene encoding a transcription factor.

Aoyama et al. teach a glucocorticoid-mediated transcriptional induction (GVG) system that can be used in plants. The system employs the transcription factor, GVG, which consists of the hormone binding domain of the rat glucocorticoid receptor, the DNA-binding from the yeast transcription factor GAL4, and the trans-activating domain from the herpes viral protein, V16. The GVG gene was introduced into tobacco with a luciferase gene wherein the promoter comprises six copies of the GAL4 upstream activating sequence (UAS). Luc expression was

induced upon treatment with dexamethasone. Also taught are advantages of the system, including that glucocorticoid is non-toxic to plants; glucocorticoid can easily permeate plant cells, resulting in rapid gene induction; that the induction level can be regulated by using different concentrations of glucocorticoid, which allows one to examine dose-dependent effects of induced gene products (pages 606-610).

It would have been obvious and within the scope of one of ordinary skill in the art at the time the invention was made to modify the method of marker gene excision of Sugita et al. by using any inducible promoter system to induce the recombinase gene, including the GVG system of Aoyama et al. It would have been obvious to place the recombinase gene under the control of the inducible promoter containing the UAS sequences of Aoyama et al. It would also have been obvious to place the transcription factor gene within the segment of the vector of Sugita et al. that is removed by the recombinase, as it would no longer be required after the removal of the promoter that it regulates. One would be motivated to use the GVG system given its advantages taught by Aoyama et al., and given that the glucocorticoid system promoter was cited by Sugita et al.

11. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sugita et al. (U.S. Patent No. 6,326,192) in view of Aoyama et al. (Plant J., 1997, Vol. 11, pages 605-612) as applied to claims 39-44, 59, 60, and 72 above, and further in view of Albert et al. (Plant J., 1995, Vol. 7, pages 649-659).

The claims are broadly drawn towards any vector comprising a gene of interest, a marker gene, a transcription factor gene, an inducible gene encoding a recombinase, and two

recombination sites, wherein the recombination sites flank the transcription factor gene, marker gene and the inducible gene; or a method for excising a marker gene from the genome of a transgenic plant or plant cell, comprising transfecting a plant or plant cell with said vector; or a plant or plant cell comprising said vector; or wherein said recombination sites are mutant lox sites and have a lower affinity for CRE than does wild-type lox.

Sugita et al. in view of Aoyama et al. teach a method for maker gene excision in plants, as discussed above.

Sugita et al. in view of Aoyama et al. do not teach mutant lox sites.

Albert et al. teach mutant lox sites that require higher concentrations of Cre recombinase than wild-type lox sites (pages 650-651).

It would have been obvious and within the scope of one of ordinary skill in the art at the time the invention was made to further modify the method for marker gene excision of Sugita et al. in view of Aoyama et al. by using mutant lox sites and the Cre recombinase of Albert et al. One would be motivated to use the mutant lox sites, as they are still recognized by the Cre recombinase.

12. No claim is allowed.

Contact Information

Any inquiry concerning this or earlier communications from the examiner should be directed to Ashwin Mehta, whose telephone number is 703-306-4540. The examiner can normally be reached on Mondays-Thursdays and alternate Fridays from 8:00 A.M to 5:30 P.M.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Amy Nelson, can be reached at 703-306-3218. The fax phone numbers for the organization where this application or proceeding is assigned are 703-305-3014 and 703-872-9306 for regular communications and 703-872-9307 for After Final communications. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

A.M.
February 14, 2002

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